

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES ON DERIVATIVES OF  
SULFANILAMIDE AND SULFADIAZINEN. Elangovan<sup>1</sup>, S. Sowrirajan<sup>2</sup> and T. Kolochi<sup>1\*</sup>

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## ABSTRACT

Derivatives of sulfanilamide and sulfadiazine were prepared and characterized by analytical data, IR, <sup>1</sup>HNMR, <sup>13</sup>CNMR, Fluorescence, UV-Vis spectra and were screened for anti-bacterial activities against gram - positive bacteria *Staphylococcus aureus* and gram - negative bacteria *Escherichia coli*, *Klebsiella aerogenes* and *Bacillus subtilis* and anti-fungal activities against *Aspergillus niger* and *Candida albicans* by disc diffusion method. Ciprofloxacin and Nystatin were used as standard drug for bacteria and fungi.

**KEYWORDS:** Sulfanilamide, Sulfadiazine, 4-chlorobenzaldehyde, 3-hydroxybenzaldehyde, Zone of inhibition, Anti-bacterial activities, Anti-fungal activities, Ciprofloxacin, Nystatin.

## INTRODUCTION

One or more carbon nitrogen bonds, carbon oxygen bonds or carbon sulphur bonds containing heterocyclic organic compounds play an important role in pharmaceutical chemistry.<sup>[2,3]</sup> Quinine,<sup>[4,5]</sup> Sulfathiazole, Sulfadiazine,<sup>[6-9]</sup> Sulfapyridine, Sulfasalazine, Sulfaquinoxaline, Silver Sulfadiazine,<sup>[10]</sup> Sulfamethoxazole<sup>[11]</sup> and chloroquine are carbon nitrogen, carbon oxygen and carbon sulphur bonded heterocyclic organic compounds which are used to inhibit the growth of *Escherichia coli*, *Klebsiella aerogenes*, *Staphylococcus aureus*, *Bacillus subtilis*, *Aspergillus niger* and *Candida albicans* in the human body, because they are analogs of the 4-amino benzoic acid. Recently in a study, imine compounds have shown that they are highly active against bacteria and fungi. In the present work we prepared imine compounds from 4-chlorobenzaldehyde, 3-hydroxybenzaldehyde and sulfanilamide or sulfadiazine to inhibit the growth of tested bacteria and fungi by nucleophilic addition reaction forming a hemiacetal followed by a hydration to generate azomethine.<sup>[17,18]</sup>

## MATERIALS AND METHODS

## Materials

All the reagents used were of AR grade. Commercially available rectified spirit was dried over anhydrous quicklime for 24 hours, filtered and distilled before use (BP 78°C). Dimethylsulphoxide and N,N-dimethylformamide were used as such 4-chlorobenzaldehyde, 3-hydroxybenzaldehyde,

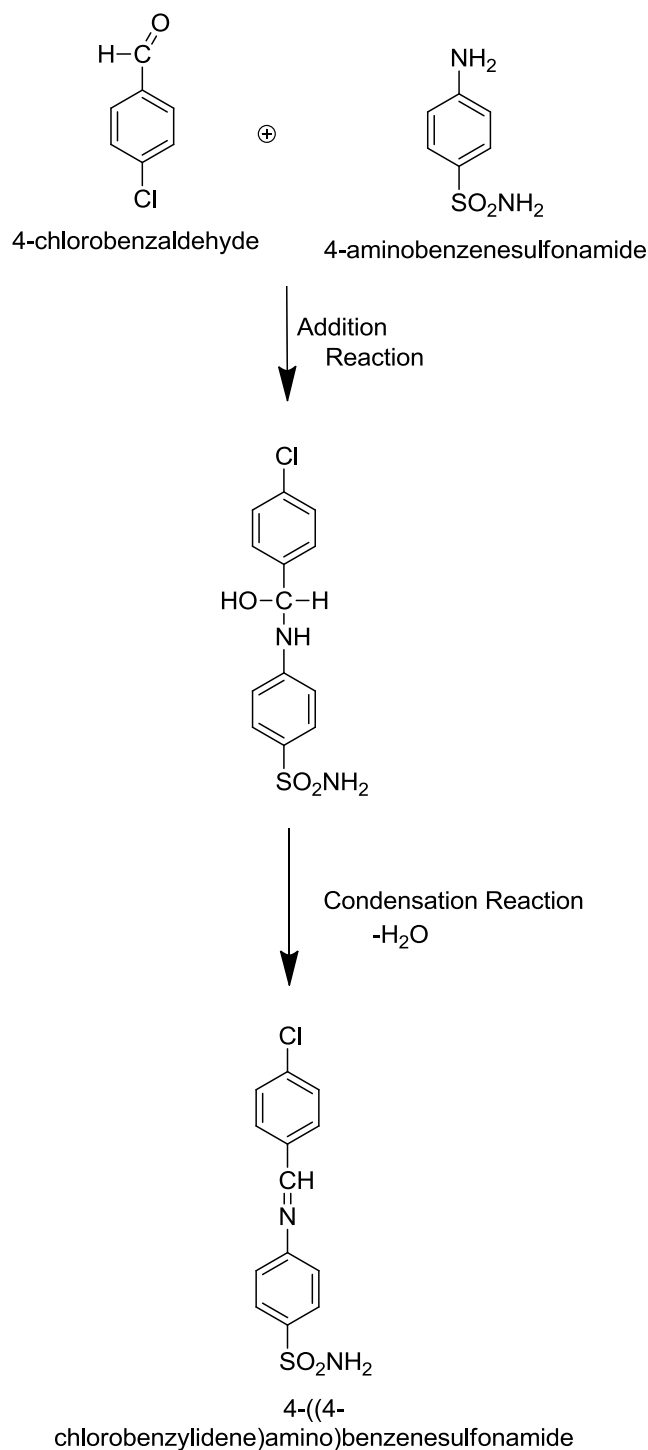
sulfadiazine and sulfanilamide were purchased from Alfa Aser.

## Instruments

Melting points were determined using Elico melting point apparatus. Elemental analysis (C, H, N, O, S, I) were performed using Elementar Vario EL III. IR spectra of the compounds were recorded in KBr pellets with Cary 630 FTIR Spectrometer in the 4000-400 cm<sup>-1</sup> range. The <sup>1</sup>H NMR spectra were recorded on a Bruker 400 MHz FT-PMR Spectrometer. The electronic spectra were recorded in Cary series UV-Vis spectrophotometer in the 200-800 nm range.

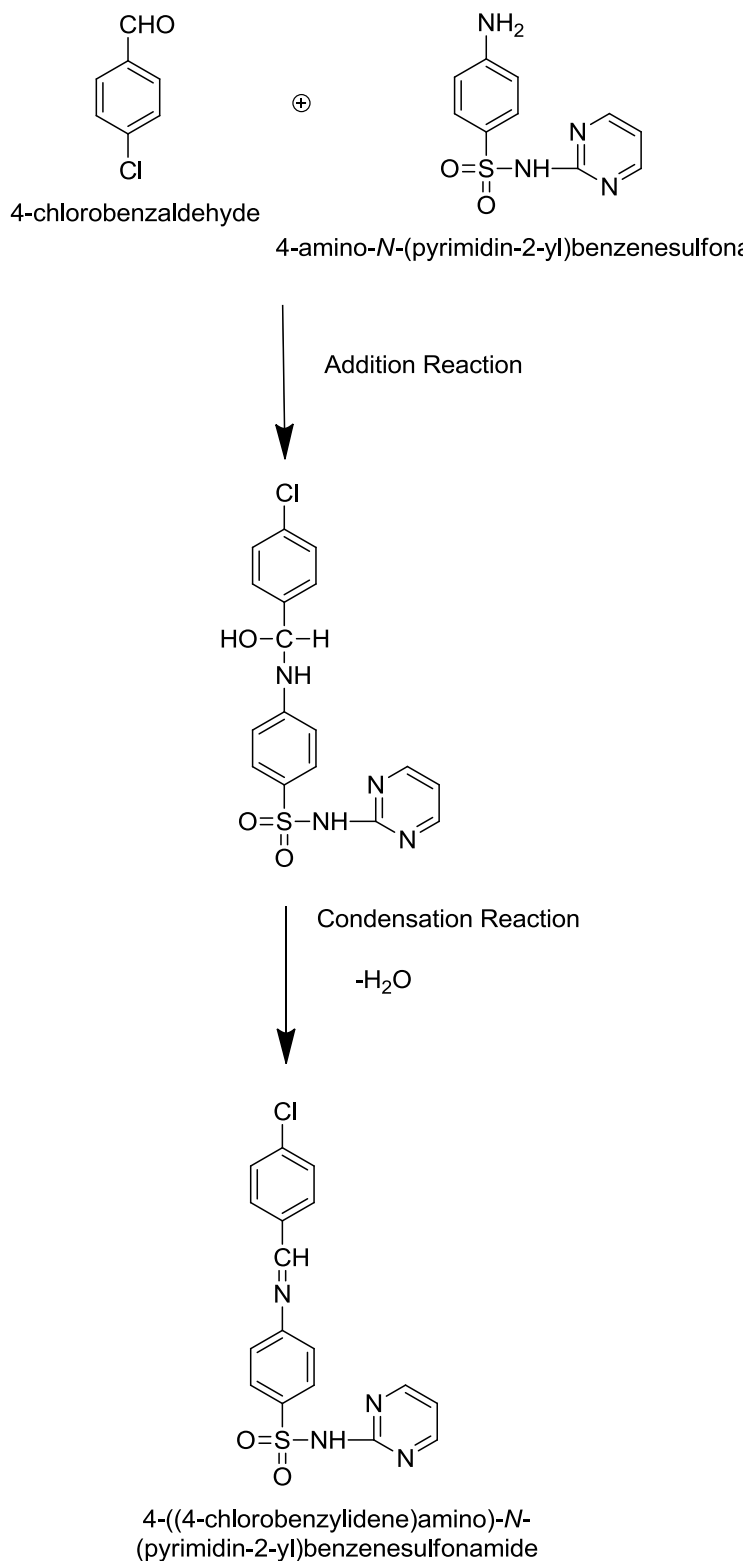
**Preparation of 4-((4-chlorobenzylidene)amino)benzenesulfonamide(I).**

1.72gm of sulfanilamide (0.01mol) was mixed with 1.40gm of 4-chlorobenzaldehyde(0.01mol) and was grained well in acidic acid medium at room temperature. The mixture was transferred into hundred milliliter Round Bottom flask and was refluxed for six hours in oil bath. The solid product 4-((4-bromobenzylidene)amino)-2-hydroxybenzoic acid was filtered and washed with ethanol and recrystallized in DMSO and then dried over vacuum desiccator.<sup>[12]</sup>

**Scheme 1**

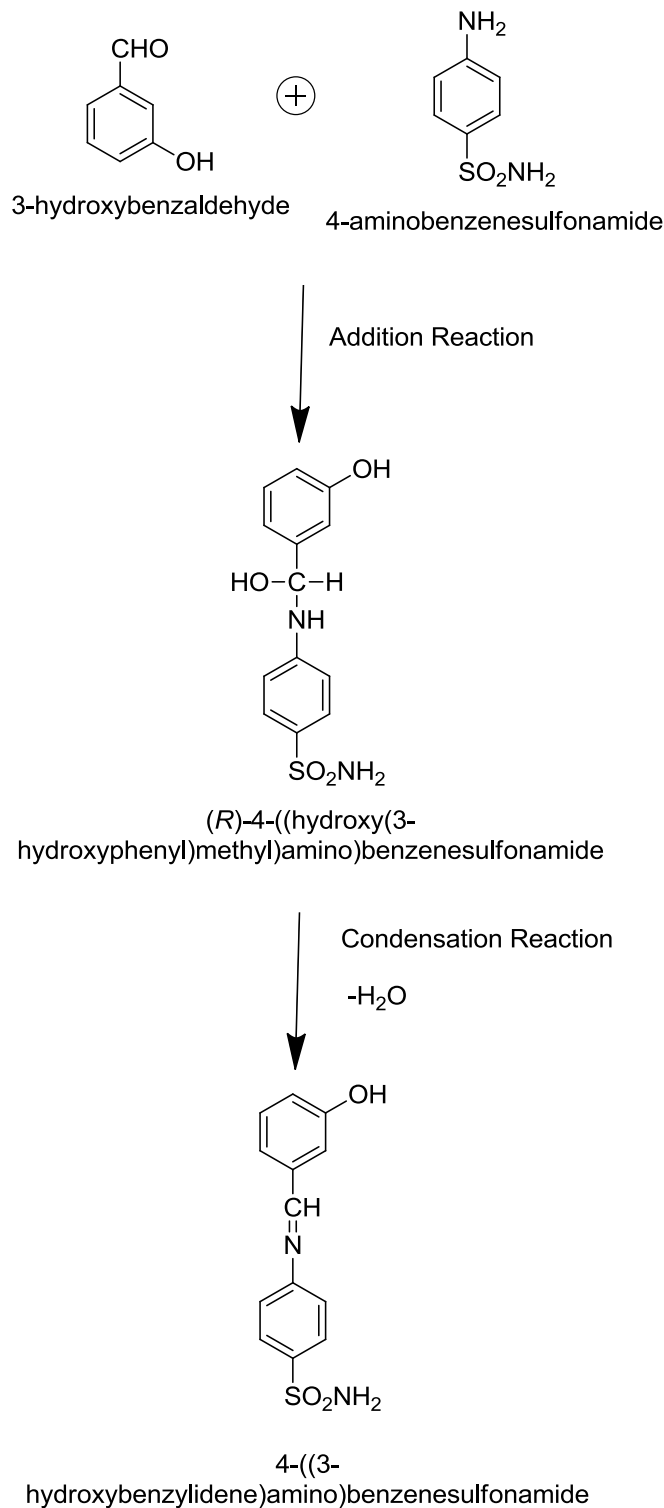
**Preparation of 4-((4-chlorobenzylidene)amino)-N-(pyrimidin-2-yl)benzenesulfonamide(II)**

A mixture of sulfadiazine (2.50gm,0.01mol) and 4-chlorobenzaldehyde (1.40gm,0.01mol) was grained in a mortar with a pestle made of porcelain for 10 minutes. The mixture turned pasty after few minutes of grinding. It was grained till brown Colour product appears. The mixture was left overnight. The resultant product 4-((4-chlorobenzylidene)amino)-2-hydroxybenzoic acid was recrystallized using ethanol and then dried over vacuum desiccator.

**Scheme 2**

**3.4.3-Preparation of 4-((3-hydroxybenzylidene)amino)benzenesulfonamide(III)**

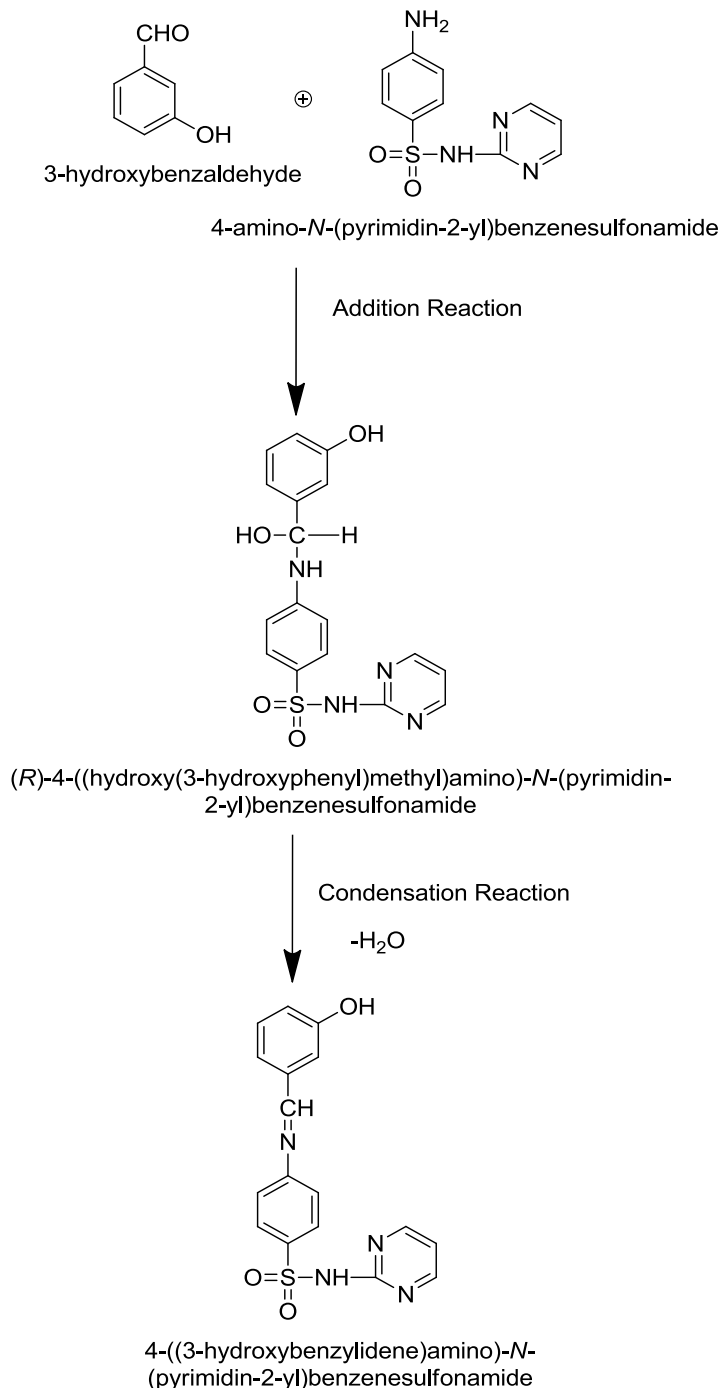
Equimolar quantities of sulfanilamide (1.72mg,0.01mol) and 3-hydroxybenzaldehyde (1.22mg,0.01mol) were dissolved in 20 ml of DMSO and 3 drops of glacial acetic acid was added and refluxed for 3 hours. After completion of the reaction (monitored by TLC), some solvent was distilled out, the reaction mixture was poured on ice cold water and the solid 4-((3-hydroxybenzylidene)amino)benzenesulfonamide came out which was filtered and then recrystallized by DMSO and then dried over vacuum desiccator.



Scheme 3

### 3.4.4-Preparation of 4-((3-hydroxybenzylidene)amino)-N-(pyrimidin-2-yl) benzenesulfonamide(IV).

4-((3-hydroxybenzylidene)amino)-N-(pyrimidin-2-yl) benzenesulfonamide(IV) was prepared from equimolar quantity of sulfadiazine (2.50mg,0.01mol) and 3-hydroxybenzaldehyde (1.22mg,0.01mol) in 30 ml of methanol were heated at 70°C on water bath for 4-hrs in presence of few drops of glacial acetic acid. The crude product were obtained after removal of methanol under reduced pressure. The products were recrystallized from methanol and then dried over vacuum desiccator.



**Scheme 4**

### Antimicrobial susceptibility test by Disc diffusion Technique

#### Principle

Disc impregnated with known concentration of antibiotics are placed on an agar plate that has been

inoculated uniformly over the entire plate with a culture of the bacterium to be tested. The plate is incubated for 18 to 24 hours at 37°C. During this period, the antimicrobial agent diffuses through the agar and may prevent the growth of the organism. Effectiveness of

susceptibility is proportional to the diameter of the inhibition zone around the disc. Organisms which grow up to the edge of the disc are resistant.

#### Procedure

The plate was labeled with the name of the culture, sample and standard at the bottom of the plate. Then sterile cotton swab on a wooden applicator stick was dipped into the bacterial suspension. Excess fluid was removed by rotating the swab and rubbed gently over the plate to obtain uniform distribution of the inoculums. The sterile disc was held on the inoculated plate with the help of micropipette. The sample was leveled in the sterile disc and incubated at 37°C in an incubator. After incubation, the diameter of the zone of inhibition of growth was measured.

#### (I)4-((4-chlorobenzylidene)amino)benzenesulfonamide.

FTIR (cm<sup>-1</sup>) = 1625(CH=N), 650(C-Cl), 3302(S-NH)

<sup>1</sup>H NMR & (ppm) = 8.3(CH), 1.9(NH)

<sup>13</sup>C NMR & (ppm) = 160(CH), 136.5(C-Cl), 134.3(C-CH), 155.1(C-N)

UV-Vis (λ<sub>max</sub>) = 275nm

Fluorescence Spectra (λ<sub>max</sub>) = 339nm

#### (II)4-((4-chlorobenzylidene) amino) – N -(pyrimidin-2-yl)benzenesulfonamide

FTIR (cm<sup>-1</sup>) = 1651(CH=N), 650(C-Cl), 3358(S-NH)

<sup>1</sup>H NMR & (ppm) = 8.3(CH), 3.9(NH)

<sup>13</sup>C NMR & (ppm) = 160(CH), 136.3(C-Cl), 169.1(C-NH), 155.1(C-N)

UV-Vis (λ<sub>max</sub>) = 250nm

Fluorescence Spectra (λ<sub>max</sub>) = 338nm

#### (III)4-((3-hydroxybenzylidene)amino)benzenesulfonamide

FTIR (cm<sup>-1</sup>) = 1625(CH=N), 3337(OH), 3481(S-NH)

<sup>1</sup>H NMR & (ppm) = 8.3(CH), 1.9(NH), 5.3(OH)

<sup>13</sup>C NMR & (ppm) = 160(CH), 158.4(C-OH), 169.3(C-NH), 155.1(C-N)

UV-Vis (λ<sub>max</sub>) = 231nm, 362nm

Fluorescence Spectra (λ<sub>max</sub>) = 371nm

#### (IV)4-((3-hydroxybenzylidene)amino)-N-(pyrimidin-2-yl) benzenesulfonamide

FTIR (cm<sup>-1</sup>) = 1669(CH=N), 3213(OH), 3336(S-NH)

**Table-1.**

Observation	Report
Inhibition zone > 15mm	Highly active
Inhibition zone > 10mm	Moderately active
Inhibition zone > 5mm	Slightly active
Inhibition zone ≤ 5mm	Inactive

#### Observation of antibacterial and antifungal activities.

#### RESULTS AND DISCUSSION

The physical character and analytical data of the derivatives of sulfa drugs I,II,III and IV are shown in table.2.

The nature of bonding and structure of I,II,III and IV are elucidated by the elemental analysis, melting point, FTIR, <sup>1</sup>HNMR, <sup>13</sup>CNMR, spectral analysis, chromatography and molar ratio methods.

$^1\text{H NMR}$  & (ppm) = 8.3(CH), 3.9(NH), 5.3(OH)

$^{13}\text{C NMR}$  & (ppm) = 160(CH), 158.4(C-OH), 169.2(C-NH), 155.1(C-N)

UV-Vis ( $\lambda_{\text{max}}$ ) = 215nm, 319nm

Fluorescence Spectra ( $\lambda_{\text{max}}$ ) = 351nm

#### Antibacterial bioassay

Antibacterial activities<sup>[13,14]</sup> of derivatives of sulfa drugs were screened against bacterial species like gram positive bacteria *Staphylococcus aureus*, and gram negative bacteria *Escherichia coli*, *Klebsiella aerogenes* and *Bacillus subtilis* by disc diffusion method<sup>[10]</sup> and the results obtained are formulated in Table.3 and Fig. 1-4. The test was carried out in DMSO solution at a concentration of 100ppm using Muller Hinton agar media. Ciprofloxacin was used as the standard drug.

#### Antifungal bioassay

Antifungal<sup>[15,16]</sup> screening of derivatives of sulfa drugs were carried out against *Aspergillus niger* and *Candida albicans* by disc diffusion method and the results obtained are formulated in Table.3 and Fig.5 & 6. The test was carried out in DMSO solution at a concentration of 100 ppm. Results were compared with standard drug Nystatin at the same concentration.

Table-2.

S. NO	MF	MW	COLOUR	MP	YIELD	ELEMENTAL ANALYSIS						
						C	H	O	N	S	Cl	M/Z
1	C <sub>13</sub> H <sub>11</sub> ClN <sub>2</sub> O <sub>2</sub> S	294.76	Pale Yellow	182	90%	52.92	3.73	10.86	9.50	10.86	11.87	99.74
2	C <sub>17</sub> H <sub>13</sub> ClN <sub>4</sub> O <sub>2</sub> S	372.83	Yellow	228	85%	54.72	3.48	8.58	15.02	8.58	9.38	99.75
3	C <sub>13</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub> S	276.31	Pale Yellow	130	92%	56.45	4.34	17.37	10.13	11.58	---	99.87
4	C <sub>17</sub> H <sub>14</sub> N <sub>4</sub> O <sub>3</sub> S	354.38	Yellow	110	87%	57.56	3.95	13.54	15.80	9.02	---	99.87

The physical and analytical data of azomethine compounds I, II, III and IV.

Table-3.

S.No	Name of the Microorganism	Zone of inhibition				S. control	Std.
		I 201641	II 201645	III 201644	IV 201640		
1	<i>Escherichia coli</i>	20	12	14	-	-	38
2	<i>Klebsiella aerogenes</i>	26	18	13	-	-	30
3	<i>Bacillus subtilis</i>	30	15	16	08	-	40
4	<i>Staphylococcus aureus</i>	20	22	12	08	-	35
5	<i>Aspergillus niger</i>	16	12	13	18	-	35
6	<i>Candida albicans</i>	16	15	23	21	-	32

Antibacterial and antifungal activities of azomethine compounds I, II, III and IV.

(I) 4-((4-chlorobenzylidene)amino)benzenesulfonamide,

(II) 4-((4-chlorobenzylidene)amino)N-(pyrimidin-2-yl)benzenesulfonamide,

(III) 4-((3-hydroxybenzylidene)amino)benzenesulfonamide and

(IV) 4-((3-hydroxybenzylidene)amino)-N-(pyrimidin-2-yl)benzenesulfonamide.

Antibacterial activities of azomethine compounds I, II, III and IV (Fig. 1 – 4).



Fig. 1

*Klebsiella aerogenes*

Fig. 2

*Escherichia coli*

Fig. 3

*Staphylococcus aureus*

Fig. 4

*Bacillus subtilis*

Antifungal activities of azomethine compounds I,II,III and IV(Fig. 5 & 6)



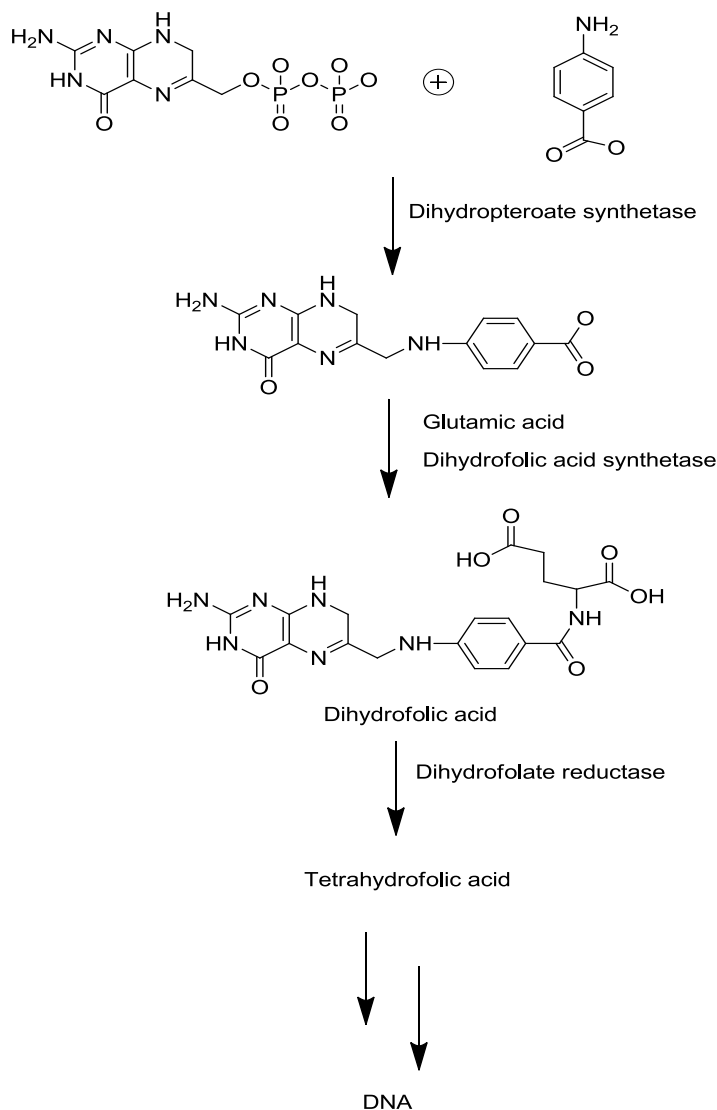
**Fig. 5**  
*Aspergillusniger*

**Fig. 6**  
*Candida albicans*

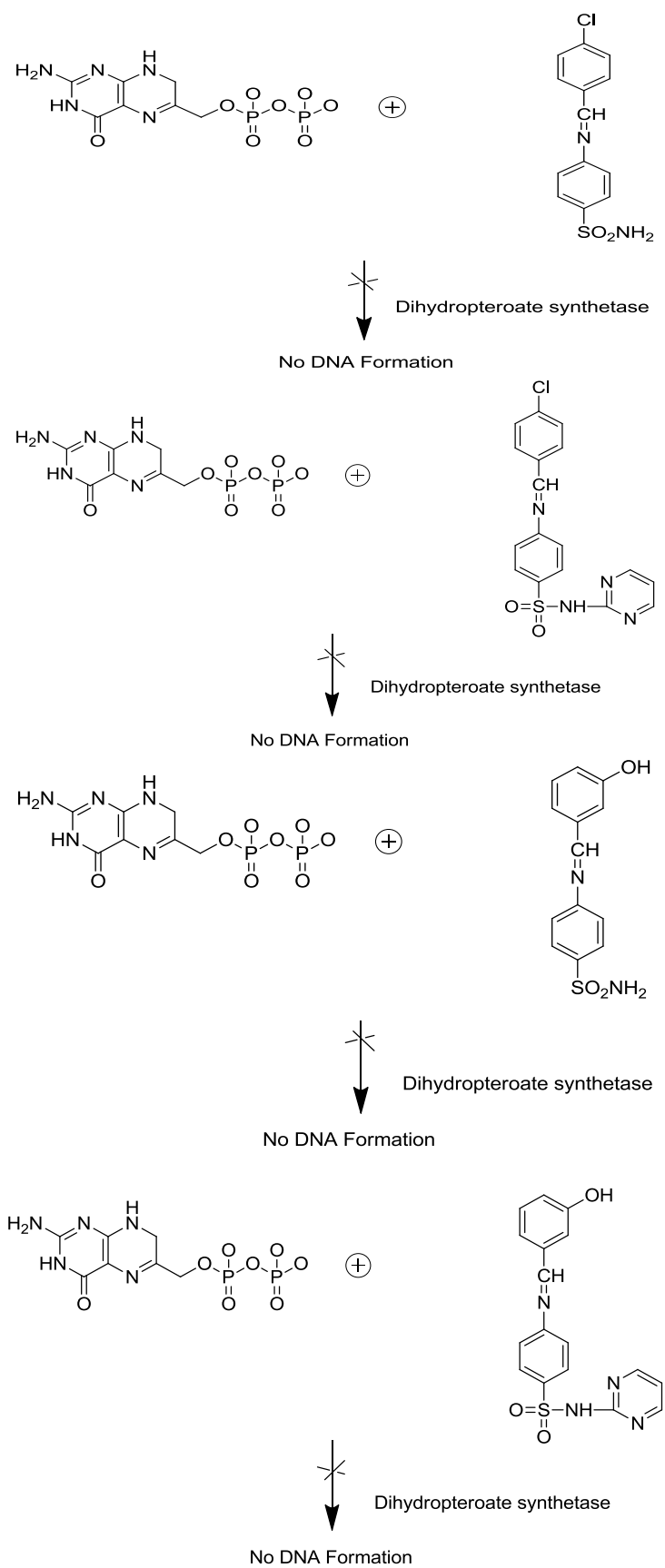
The antibacterial and antifungal activity of azomethine compounds I,II,III and IV (table. 3 and figure 1-6) clearly indicate that they inhibit the growth of tested bacteria and fungi in the decreasing order I>II>III>IV.

Azomethine compounds I,II,III and IV prevent bacterial reproduction by acting as an antimetabolite to para-aminobenzoic acid (PABA), where PABA is a vital component in the biosynthesis of tetrahydrofolic acid. Competitive inhibition of PABA processing enzymes by

I,II,III and IV ultimately blocks the action of dihydrofolic acid synthetase, and therefore prevents dihydrofolic acid formation (Scheme 5). As bacteria are unable to take up tetrahydrofolic acid from their surroundings, inhibition of dihydrofolic acid synthetase will starve the bacteria of thymidine and uridine. These two nucleosides are required for DNA replication and transcription, therefore cell growth and division is disrupted, and thus provides enough time for the body's own immune system to eliminate the bacterial threat.<sup>[17]</sup>







Scheme 5

## CONCLUSION

Azomethine compounds(I)4-((4-chlorobenzylidene)amino)benzenesulfonamide,(II)4-((4-chlorobenzylidene) amino) – N -(pyrimidin-2-yl)benzenesulfonamide, (III)4-((3-hydroxybenzylidene)amino)benzenesulfonamide and (IV)4-((3-hydroxybenzylidene)amino)-N-(pyrimidin-2-yl) benzenesulfonamide were prepared and were screened against bacteria and fungi and had shown that chlorosubstituted derivatives of sulfonamides were more active against bacteria and fungi than other derivatives of sulfonamides.

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